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Persistence of selected ammonium- and phosphonium-based ionic liquids in urban park soil microcosms

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Abstract

Knowledge about biodegradability of ionic liquids (ILs) in terrestrial systems is limited. Here, using urban park soil microcosms spiked with either ammonium- or phosphonium-based ILs [didecyldimethylammonium 3-amino-1,2,4-triazolate, benzalkonium 3-amino-1,2,4-triazolate, trihexyl(tetradecyl)phosphonium chloride, or trihexyl(tetradecyl)phosphonium 1,2,4-triazolate], we studied their (i) 300-day primary biodegradation, and (ii) influence on CO_2 evolution from the microcosms. The primary biodegradation ranged from 21 to 33% of total compound in the dissolved phase. The evolution of CO_2 from spiked microcosms was either lower or within the range of background soil respiration, indicating no or small mineralization of the parent

compounds and/or their metabolites, and their negligible or small toxicity to soil microorganisms. Our results suggest the potential for persistence of the four studied ILs in urban park soils.

Keywords: impact assessment; ionic liquids; risk assessment; terrestrial toxicity; biotransformation;

1. Introduction

Ionic liquids (ILs) are a group of chemicals composed of an organic cation and an organic or inorganic anion. Due to a large number of cation - anion combinations, the number of ILs which may be obtained is tremendous (up to 10^{18}) (Rogers and Seddon 2003). Various ILs are currently applied in catalysis and biocatalysis, and in organic synthesis (e.g., multi-ton scale use of imidazolium-based IL in the BASIL™ process developed by BASF) (Plechkova and Seddon 2008; Quijano et al. 2010). Although there are no reports providing information about the presence of ILs in water or soil ecosystems yet, the increasing use of ILs in the industry is expected to lead to environmental pollution. Consequently, there has been an increasing number of studies dealing with ILs behaviour and impact on the environment (Ranke et al. 2007; Petkovic et al. 2010; Pham et al. 2010; Cvjetko Bubalo et al. 2014).

Low volatility of ILs excludes emission into air as an exposure route, but there can be direct release routes to freshwater or soil. It is generally thought that relatively high thermal and chemical stability of ILs contributes to their low biodegradability (Quijano et al. 2011; Neumann et al. 2012), primarily on aquatic environments (Coleman and Gathergood 2010; Ford et al. 2010; Stolte et al. 2011). In soils, Modelli et al. (2008), demonstrated that at the end of a 180-day respirometric experiment, none of the four imidazolium-based ILs was mineralized completely, and the amount of CO_2 evolved depended on both the type of cation and the type of anion. Primary biodegradation, defined as an alteration in the chemical structure of a substance, brought about by biological action, resulting in the loss of a specific property of that substance (OECD 301, 1992), has been studied and formation of biotransformation products has also been documented (Stolte et al. 2008; Pham et al. 2009; Docherty et al. 2010). Sorption of ILs onto the soil matrix can occur, depending on soil parameters (such as organic carbon content or cation exchange capacity), which can lead to a decrease of their bioavailability and biodegradability and contribute to the potential persistence (Stepnowski et al. 2005; Studzińska et al. 2008; Mrozik et

al. 2012). Most published studies focused on toxicity of ILs to single, isolated microbial strains (Pham et al. 2010; Cvjetko Bubalo et al. 2014), but little is known about the influence of ILs on the activity of soil microbiota. Recently, Peric et al. (2014), showed that aprotic ILs inhibited soil respiration in the concentration range from 100 mg kg^{-1} to 10,000 mg kg^{-1} depending on the ILs structure.

The aim of our study was to investigate primary biodegradation of ammonium- and phoshonium-based ILs in urban park soil microcosms, simulating an accidental spill, and their influence on CO_2 evolution from the microcosms. The four ILs studied were: (i) didecyldimethylammonium 3-amino-1,2,4-triazolate ([DDA][3AT]), (ii) benzalkonium 3-amino-1,2,4-triazolate ([BDA][3AT]), (iii) trihexyl(tetradecyl)phosphonium chloride ($[P_{66614}][Cl]$), and (iv) trihexyl(tetradecyl)phosphonium 1,2,4-triazolate ($[P_{66614}][Tr]$). Apart from $[P_{66614}][Cl]$, the studied ILs have antifungal properties. The triazole-based ILs were previously described as novel anti-microbial and anti-fungal plant protection agents with a broad spectrum of activity (Walkiewicz et al. 2010), and are thus expected to be toxic to the microorganisms present in the soil. An urban park soil of known biodegrading potential toward petroleum hydrocarbons (Sydow et al. unpublished results) was employed. The primary biodegradation (300-day) was studied by measuring ILs' residues in the soil, while CO_2 evolution from spiked microcosms was measured using a respirometric test.

2. Materials and methods

2.1. Synthesis of ionic liquids

The ammonium-based ILs were synthesised with the use of quartemary ammonium bromides, which were obtained in reaction of equimolar amount of amine (decyldimethylamine or benzyldimethylamine) with appropriate bromoalkane (1-bromodecane or 1-bromododecane respectively). The reaction was carried out using anhydrous acetonitrile as solvent at 70ºC for 24 h. After cooling the reaction mixture to room temperature, quaternary ammonium bromides were precipitated by adding cold ethyl acetate. The bromides were filtered and dried under vacuum at 50ºC. Product yield was over 95%. Next, 3-amino-1,2,4-triazolate ILs were prepared by *in situ* deprotonation of corresponding azole with sodium or potassium hydroxide in methanol and mixed with an equimolar amount of appropriate quaternary ammonium bromides, according to

the method described in Walkiewicz et al. (2010). The phosphonium-based ILs were prepared according to method described by Cieniecka-Rosłonkiewicz et al. (2005). Briefly, trihexyl(tetradecyl)phosphonium chloride was prepared in reaction of trihexylphosphine and 1-bromotetradecane. The 1,2,4-triazolate was obtained by *in situ* deprotonation of corresponding azole with sodium hydroxide in methanol, followed by reaction with chloride precursor. Water content of the synthesized ILs was determined by Karl Fisher method for all prepared compounds, and was less than 0.1%. The list of the studied ILs is given in Table 1.

2.2. Soil

The soil used in the experiments was collected from a city park in Poznań, Poland (N 52.4011445, E 16.9222993) and according to Unified Soil Classification System, was characterized as fine grained silt loam type OL belonging to organic silts and organic silty clays of low plasticity. The composition of experimental soil was as follows: clay 4 ± 1 [%]; silt 83 ± 3 [%]; sand 13 ± 2 [%]. Detailed characteristics of the soil: organic carbon 5.44 ± 0.31 [g kg^{-1}]; nitrogen 0.57 ± 0.07 [g kg^{-1}]; phosphorous 0.080 ± 0.005 [g kg^{-1}]; pH 6.95 ± 0.7; bulk density 1.41 ± 0.06 [Mg m^{-3}]; porosity 0.455 ± 0.03 [m^3 m^{-3}]; moisture 18 ± 1 [%]; cation exchange capacity 22.1 ± 0.8 [$cmol_c$ kg^{-1}]. A sign ± represents standard deviation (n = 3).

2.3.1. Primary biodegradation

Each primary biodegradation experiment was set up with four replicates. Three replicates were used to analyse ions residues with the use of HPLC-MS, while one replicate was used for moisture monitoring over the course of the experiment. The samples were prepared as follows: 10 g of non-sterilized soil was added into bottles and then spiked with a methanol solution of each IL to reach an approximate concentration of 4000 mg kg^{-1} of IL per sample. The concentration in this range is typically used in hydrocarbon biodegradation tests (Lisiecki et al. 2014). Next, methanol was evaporated with nitrogen. Afterwards, untreated soil in the amount of 90 grams was added. The soil was later vigorously mixed. Finally, the microcosms were incubated at 20°C for 300 days. The soil moisture was determined once a week by using moisture analyser (RADWAG MA 110.R, Poland), and adjusted to 18% if necessary. After 300 days three 0.5 g samples of each bottle replicate (giving nine sampling points for each studied IL) were subjected to three-step ultrasound assisted extraction with methanol (3 x 1 mL) and analysed by HPLC-MS

to determine the residual masses of ILs ions. Controls were prepared as above, except that the urban soil was previously sterilized in order to investigate the influence of sorption on primary biodegradation of the compounds. The soil was divided into aliquots of 30 g, frozen, placed in sealed polyethylene bags and irradiated at 40,000 grey using a ^{192}Ir source (Alef and Nannipleri 1995). Afterwards, a solution of selected IL was added to 10 g of sterilized soil. Next methanol was evaporated and untreated sterilized soil in the amount of 90 grams was added and samples were mixed vigorously. After 300 days of incubation under sterile conditions, soil from each replicate was used in order to determine the fraction of ILs that was not sorbed onto soil matrix. For this purpose three 0.5 g samples of each bottle replicate (giving nine samples in total) were subjected to three-step ultrasound assisted extraction with methanol (3 x 1 mL) and analysed by HPLC-MS.

The primary biodegradation of selected ILs was calculated with regard to initial masses of ILs at the beginning of the experiment, residual masses of cations and anions at the end of the experiment and percentage values of ILs fraction sorbed onto studied soil. It was not possible to determine the residual anion amount for [P_{66614}][Tr], since the employed analytical method did not allow it. The residues of [Cl] anion were not analysed, since halide ILs undergo hydrolysis reaction in the environment. Therefore, in the case of both phosphonium-based ILs primary biodegradation was calculated ignoring the final mass of the anion. Thus, primary biodegradation of ammonium- and phosphonium-based ILs cannot be compared directly. The primary biodegradation was calculated as presented in Eq. 1.

$$PB = 100\% - \frac{M_C \cdot X_C + M_A \cdot X_A}{M_{IL}} - FS_{IL} \qquad \text{(Eq. 1)}$$

where:

PB – primary biodegradation of IL [%];

M_C – residual mass of cation at the end of the experiment [g];

M_A – residual mass of anion at the end of the experiment [g];

M_{IL} – initial mass of IL at the beginning of the experiment [g];

X_C – percentage of molar mass of cation in overall molar mass of IL [%];

X_A – percentage of molar mass of anion in overall molar mass of IL [%];

FS_{IL} – the sorbed fraction of IL onto soil matrix [%].

The sorbed fraction of each IL was calculated with regard to mass of extracted IL and mass of introduced IL as presented in Eq. 2.

$$FS_{IL} = \left(1 - \frac{M_E}{M_I}\right) \cdot 100\% \qquad \text{(Eq. 2)}$$

where:

M_E – mass of extracted IL [g];

M_I – mass of introduced IL [g].

2.3.2. HPLC-MS analysis

Three 1 mL soil extracts of each sampling point (obtained via three-step extraction of each 0.5 g soil sample) were combined, filtered through a 0.2 µm PTFE syringe filter and diluted with methanol : water solution (80:20 v/v). The HPLC-MS analyses were performed with the UltiMate 3000 RSLC chromatograph from Dionex (Sunnyvale, CA, USA). Five µL samples were injected into a Hypersil GOLD column (100 mm × 2.1 mm I.D.; 1.9 µm) with a 2.1 mm I.D. pre-filter cartridge (0.2 µm) from Thermo Scientific (Waltham, MA, USA). The mobile phase consisted of $5x10^{-3}$ mol L^{-1} ammonium acetate in water (phase A) and methanol (phase B) at a flow rate of 0.2 mL min^{-1}. Gradient elution was performed by linearly increasing the percentage of phase B from 85 to 100% in 4 min and maintained at 100% for 3 min. The LC column effluent was directed to the API 4000 QTRAP triple quadrupole mass spectrometer from AB Sciex (Foster City, CA, USA) through the electrospray ionization source (Turbo Ion Spray) that operated in positive ion mode for analyses of cations and in negative ion mode for analyses of anions. The dwell time for each mass transition detected in the MS/MS multiple reaction monitoring mode was set to 200 ms. Nitrogen was used as curtain gas (10 psi), nebulizer gas (40 psi), auxiliary gas (45 psi) and collision gas (medium). The source temperature was 400°C and the ion spray voltage was 4500 V for cations and –4500 V for anions. The declustering potential was 50 V for cations and –50 V for anions.

2.4. Evolution of CO_2 from the microcosms

Each experiment was performed in triplicate. Microcosms were prepared as follows: 10 g of non-sterilized soil was added to 1L glass SIMAX bottles closed with PP screw caps and later spiked with a methanol solution of each IL to reach an approximate concentration of 4000 mg kg^{-1} of IL per sample. Next, methanol was evaporated with nitrogen. Afterwards, 90 grams of untreated soil were added and the soil was vigorously mixed. Finally, the microcosms were incubated at 20°C for 300 days. The amount of emitted CO_2 was determined according to respirometric tests procedure. Additionally, a set of three control replicates – bottles containing 100 g of non-sterilized urban park soil without any addition of IL – was prepared. The controls were prepared to investigate the background respiration of the used soil. Natural soil respiration was determined according to respirometric tests procedure.

The evolution of CO_2 in each soil sample was determined periodically by measuring CO_2 content in base traps (10 mL of 0.75 M NaOH in a 20-mL vial) placed in the microcosms (bottles with the used urban park soil) as described elsewhere (Szulc et al. 2014; Lisiecki et al. 2014). Each bottle contained one base trap. NaOH and Na_2CO_3 from each trap were titrated with 0.1 M HCl using an automatic titrator (Metrohm titroprocessor 686). The content of the base traps was replaced with fresh NaOH solution after each measurement.

2.5. Statistical analyses

One-way analyses of variance (ANOVA), α=0.05 were performed to detect significant differences between the treated groups and the respective controls. All of the presented error bars represent standard errors of the mean (for primary biodegradation tests n = 9, for CO_2 evolution tests n = 3).

3. Results and discussion

Below, we present results for primary biodegradation, sorption, CO_2 evolution, and carbon balance of the four studied ILs in soil microcosms. Formation of biotransformation products and potential biotransformation mechanisms, are then discussed.

3.1. Primary biodegradation and sorption

Figure 1a shows that 300-day primary biodegradation of the four studied ammonium- and phosphonium-based ILs in urban park soil microcosms was small. It reached 33 and 21% of the total compound in the dissolved phase for the two ammonium-based ILs ([DDA][3AT] and [BDA][3AT], respectively), and 25 and 29% for the two phosphonium-based ILs ($[P_{66614}][Cl]$ and $[P_{66614}][Tr]$, respectively). Figure 1b shows that the sorbed fraction of the studied ILs in soils was generally higher for the two ammonium-based ILs, reaching 44 and 64% for [DDA][3AT] and [BDA][3AT], respectively, as opposed to 13 and 29% for $[P_{66614}][Cl]$ and $[P_{66614}][Tr]$, respectively. Thus, the IL with longer alkyl chain (i.e., [BDA][3AT]) exhibited higher sorption onto soil as compared to the shorter-chain alternative (i.e., [DDA][3AT]), and furthermore the IL with inorganic anion (i.e., $[P_{66614}][Cl]$) is the least sorbed among the four studied ILs. Both findings are in agreement with Studzinska et al. (2008) who showed how sorption of ILs onto soil depends on the side chain length and on the cation-anion combination. In soils, biodegradation of organic compounds can be influenced by their bioavailability for microbial uptake from the dissolved phase (Alexander 2000). However, differences in sorption between the studied ILs do not translate directly into apparent differences in primary biodegradation, suggesting that the persistence of the four ILs is rather due to their low inherent biodegradability than due to bioavailability restrictions in the soil matrix.

3.2. Evolution of CO_2 and carbon balance

Figure 2a shows that CO_2 evolution from spiked microcosms was slightly below or within range of background soil respiration, indicating no or small negative effect of the studied ILs on respiratory activity of microbiota in the soil. Only in the case of [BDA][3AT], were statistically significant differences between the microcosms spiked with ILs and the control microcosm observed. These findings from a long-term soil microcosm experiment are not in disagreement with findings from biodegradation tests performed in aquatic systems for structurally similar ILs. In a manometric respiratory test with activated sludge, Pisarova et al. (2012) showed that one (i.e., (2-hydroxyethyl)-trimethylammonium methanesulphonate) out of three ammonium-based ILs reached 74% of ultimate biodegradation within 28 days. The two other ILs (i.e., tributyl-methylammonium methanesulphonate and butyl-trimethylammonium methanesulphonate) reached 7 and 4%, respectively. In a CO_2 headspace test, also Atefi et al. (2009) showed that

ultimate biodegradation of various tri-*n*-hexylphosphonium-based ILs in an aqueous medium after 28 days ranged from 0 to 30%, depending on the side chain functionality.

The largest CO_2 evolution was measured for the microcosms spiked with $[P_{66614}][Cl]$. We therefore performed carbon balance to see how much of the primarily biodegraded $[P_{66614}][Cl]$ could be mineralized. Even if the CO_2 evolution from that microcosms had been statistically different from that measured for the control, and assuming yield of 0.4 g of microbial carbon per 1 g of IL carbon, 63% of the primarily biodegraded $[P_{66614}][Cl]$ in the dissolved phase could be mineralized. This corresponds to 16% mineralization of total compound. For the three other ILs, measured CO_2 evolution was much lower compared to theoretical CO_2 evolution expected if all primarily biodegraded ILs had been mineralized (and assuming a yield of 0.4 g of microbial carbon per 1 g of IL) (Figure 2b). This confirms no, or very small, ultimate biodegradation of the four studied ILs in our soil microcosms and suggests that biotransformation products must have formed in the microcosms. Further, one IL (i.e., [BDA][3AT]), showed CO_2 evolution values lower as compared to the control, suggesting some, albeit small, inhibition of soil microbial activity in the microcosm spiked with this IL at total concentration of 4000 mg kg^{-1}. Peric et al. (2014) showed, that for various imidazolium-based ILs, inhibition of respiratory activity of the soil can occur in concentration range of 100 to 10,000 mg kg^{-1}, depending on the IL structure.

3.3. Potential biotransformation mechanisms

Metabolites of primary biodegradation of the studied ILs were expected to form in our microcosms, because all four ammonium- and phosphonium-based ILs have long, unbranched alkyl chains attached to the nitrogen or phosphorus atom in the organic cation. Stolte et al. (2008) already showed that ω-oxidation on the terminal methyl group in alkyl chains of imidazolium-based ILs occurs, leading to the formation of shorter-chained products with carboxylic group. Also Pham et al. (2009) and Zhang et al. (2011) showed gradual degradation of ILs' alkyl chains during biotransformation by activated sludge, or by *Pseudomonas fluorescens*, in aquatic media. In addition, hydroxylation of the aromatic ring of pyridinium-based ILs has also been reported (Docherty et al. 2010), just as in case of triazole pesticides (WHO 2006). Therefore, similar biotransformation mechanism cannot be ruled out for the three ILs containing triazolate-based anion. Short-chained metabolites of ILs biotransformation typically have lower biodegradability as compared to the parent compound (Jastorff et al. 2003; Neumann et al. 2014), which can

explain primary biodegradation of up to ca. 30% in our systems with no or small mineralization. Although some potential biotransformation products, such as aldehydes or ethanol are toxic toward microorganisms, (Stolte et al. 2008; Pham et al. 2009; Zhang et al. 2011), biotransformation products with shorter side chains and ring hydroxylation products are typically less toxic as compared to the parent compound (Docherty et al. 2010, Ranke et al. 2004; Stolte et al. 2011). This may explain why no or very small inhibition of CO_2 evolution from spiked samples occurred during the course of the biodegradation experiment.

4. Conclusions

We showed that the four studied ammonium- and phosphonium-based ionic liquids were persistent in urban park soil microcosms. Primary biodegradation ranging from 21 to 33% of total compound in the dissolved phase, combined with CO_2 evolution from the microcosms being in range of soil background respiration rate, suggest no or very small mineralization of the primary compounds and/or their potential metabolites and no or negligible toxicity to soil microorganisms.

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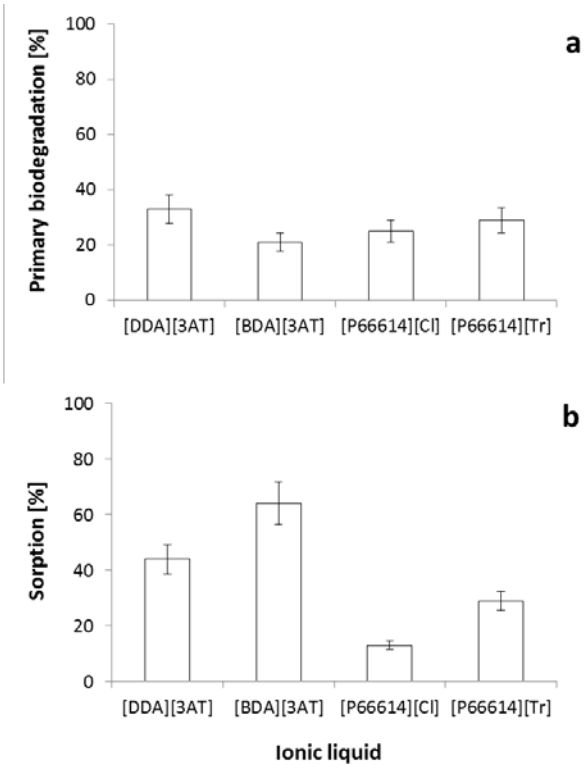
Figure captions

Fig. 1. Primary biodegradation (a), and sorption (b) of the studied ILs in soil microcosms after 300 days. [DDA][3AT] is the didecyldimethylammonium 3-amino-1,2,4-triazolate, [BDA][3AT] is the benzalkonium 3-amino-1,2,4-triazolate, [P66614][Cl] is the trihexyl(tetradecyl)phosphonium chloride, [P66614][Tr] is the trihexyl(tetradecyl)phosphonium 1,2,4-triazolate. Bars represent standard errors of the mean (n = 9). Note, that primary biodegradation of ammonium- and phosphonium-based ILs cannot be compared directly because the phosphonium-based ILs anions could not be determined (see Methods section).

Fig. 2. Evolution of CO_2 from soil microcosms over 300 days (a), and theoretical and observed CO_2 evolution at day 300 (b). The theoretical evolution is the expected CO_2 evolution if all primarily biodegraded IL had been mineralized. [DDA][3AT] is the didecyldimethylammonium 3-amino-1,2,4-triazolate, [BDA][3AT] is the benzalkonium 3-amino-1,2,4-triazolate, [P66614][Cl] is the trihexyl(tetradecyl)phosphonium chloride, [P66614][Tr] is the trihexyl(tetradecyl)phosphonium 1,2,4-triazolate. Bars represent standard errors of the mean (n = 3).


a
Primary biodegradation [%]
100
80
60
40
20
0
[DDA][3AT]
[BDA][3AT]
[P66614][Cl]
[P66614][Tr]
b
Sorption [%]
100
80
60
40
20
0
[DDA][3AT]
[BDA][3AT]
[P66614][Cl]
[P66614][Tr]
Ionic liquid

438

439 **Fig. 1.**

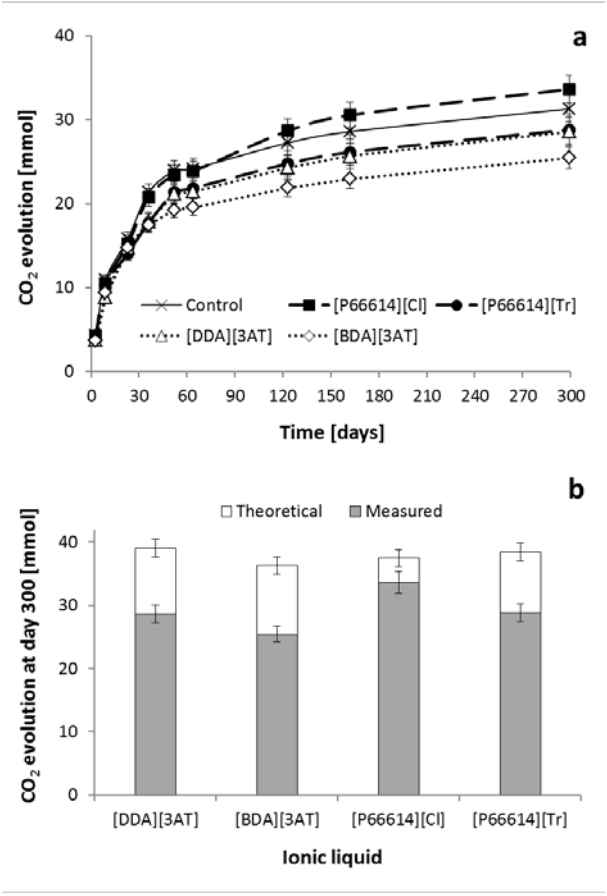

a
CO_2 evolution [mmol]
Time [days]
Control
[P66614][Cl]
[P66614][Tr]
[DDA][3AT]
[BDA][3AT]
b
Theoretical
Measured
CO_2 evolution at day 300 [mmol]
[DDA][3AT]
[BDA][3AT]
[P66614][Cl]
[P66614][Tr]
Ionic liquid

Fig. 2.

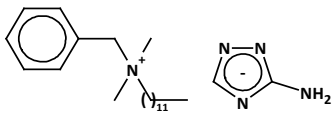
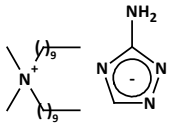
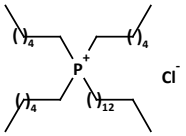
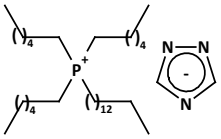
Type	Name	Acronym	Structure
Ammonium-based	didecyldimethylammonium 3-amino-1,2,4-triazolate	[DDA][3AT]	
	benzalkonium 3-amino-1,2,4-triazolate	[BDA][3AT]	
Phosphonium-based	trihexyl(tetradecyl)phosphonium chloride	[P_{66614}][Cl]	
	trihexyl(tetradecyl)phosphonium 1,2,4-triazolate	[P_{66614}][Tr]	

Table 1. Ionic liquids used during the study.

Highlights

- The studied ILs show potential for long-term persistence in soils.
- Primary biodegradation of ILs ranged from 21 to 33%.
- CO_2 evolution from the microcosms was in range of soil background respiration rate.